



RFRP Neurons – The Doorway to Understanding Seasonal Reproduction in Mammals

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Seasonal control of reproduction is critical for the perpetuation of species living in temperate zones that display major changes in climatic environment and availability of food resources. In mammals, seasonal cues are mainly provided by the annual change in the 24-h light/dark ratio (i.e., photoperiod), which is translated into the nocturnal production of the pineal hormone melatonin. The annual rhythm in this melatonin signal acts as a synchronizer ensuring that breeding occurs when environmental conditions favor survival of the offspring. Although specific mechanisms might vary among seasonal species, the hypothalamic RF (Arg–Phe) amide-related peptides (RFRP-1 and -3) are believed to play a critical role in the central control of seasonal reproduction and in all seasonal species investigated, the RFRP system is persistently inhibited in short photoperiod. Central chronic administration of RFRP-3 in short day-adapted male Syrian hamsters fully reactivates the reproductive axis despite photoinhibitory conditions, which highlights the importance of the seasonal changes in RFRP expression for proper regulation of the reproductive axis. The acute effects of RFRP peptides, however, depend on species and photoperiod, and recent studies point toward a different role of RFRP in regulating female reproductive activity. In this review, we summarize the recent advances made to understand the role and underlying mechanisms of RFRP in the seasonal control of reproduction, primarily focusing on mammalian species.

Keywords: RFRP, mediobasal hypothalamus, melatonin, seasonal reproduction, photoperiod, TSH

INTRODUCTION

Animals living in temperate and boreal latitudes experience marked seasonal changes in their environment. To overcome these environmental changes, thus increasing their chances of survival, they show seasonal changes in several aspects of their physiology, i.e., reproduction, metabolism, and behavior. Annual variations in day length are translated into an endocrine message, namely, the nocturnal secretion of the pineal hormone melatonin, which acts as a potent seasonal synchronizer of biological functions, especially reproductive activity (1–4).

The effects of photoperiod on reproductive function have long been established, and recent studies have made much progress in describing key components and pathways involved in this adaptive process. The RFamide peptide kisspeptin (kp), a very potent stimulator of gonadotropin-releasing hormone (GnRH) release, shows photoperiodic variations and was therefore thought to be a likely candidate for the photoperiodic control of reproduction in seasonal breeders, but increasing evidence now points toward another RFamide peptide, the RF (Arg–Phe) amide-related peptide

(RFRP) as the critical intermediate between the melatonin-dependent photoperiodic signal and central control of the reproductive axis (5–8).

Herein, we review our current understanding of the RFRP system in seasonal breeders, reporting the mechanisms through which melatonin impacts on RFRP synthesis and the effects of RFRPs in the seasonal control of reproduction including species-dependant and sex-specific variations in the RFRP system.

THE PINEAL HORMONE MELATONIN SYNCHRONIZES REPRODUCTION WITH SEASONS

In seasonal species, reproductive activity is restricted to a particular time of the year so that birth occurs when warmer temperatures and better accessibility to food increase the chances of survival of the offspring. Early studies have demonstrated that synchronization of reproductive activity with season is driven by the pineal hormone melatonin (2). Photoc information reaches the pineal gland *via* the retino-hypothalamo-pineal pathway that during nighttime generates a release of norepinephrine, which acts as a potent and reliable regulator of the rhythmic release of melatonin from the pineal gland. As a consequence, melatonin is synthesized and secreted in a diurnal fashion with a dramatic increase during nighttime that returns to nearly undetectable levels at daytime with the duration of elevated melatonin depending on night length (1–4, 8, 9). In long photoperiod (LP) breeders, i.e., the Syrian hamster (*Mesocricetus auratus*) that is widely used as a rodent model to study seasonal reproduction, short day lengths represented by a long nocturnal duration of melatonin secretion inhibits the reproductive axis, and removal of the melatonin signal by pinealectomy prevents this short day inhibition of reproductive activity (2). In contrast to small rodents, larger mammals with a longer gestation time, such as the sheep, are sexually active in short photoperiod (SP) and becomes sexually quiescent after transfer to LP conditions (10). Although the reproductive timing is opposite in hamsters and sheep, in both cases, the photoperiodic changes in circulating levels of melatonin synchronize reproduction with seasons.

Melatonin Modes And Sites of Action on the Reproductive Axis

Three melatonin receptor subtypes have been characterized so far; MT1 (Mel1a), MT2 (Mel1b), and Mel1c with its mammalian ortholog GPR50 (11, 12). Using the highly specific 2-[¹²⁵I]-iodomelatonin, high affinity melatonin-binding sites have been found in the hypothalamus and the *pars tuberalis* (PT) of mammals, and among species, the highest concentration of melatonin receptors is found in the PT (8, 13–16). The MT1 subtype seems to be dominantly expressed throughout species and is known to be responsible for the neuroendocrine integration of season (17, 18). Indeed, Siberian hamsters (also known as the Djungarian hamster or *Phodopus sungorus*) show seasonal reproductive responses to melatonin despite lacking a functional MT2 receptor (19). Maywood et al. found that site specific lesions of iodomelatonin-binding sites in the mediobasal hypothalamus

(MBH) prevent testicular regression in Syrian hamsters exposed to SP (20) and in Siberian hamsters, melatonin infusion into or lesions of the suprachiasmatic nucleus (SCN), alter the reproductive response to seasonal changes (21, 22). Finally, in sheep, the premammillary region of the hypothalamus contains melatonin-binding sites (23, 24) and melatonin implantations in the area of this structure, but not the PT, were shown to prevent synchronization of reproduction with photoperiod (24–27). Altogether, these data have pointed toward the potential importance of these hypothalamic regions for proper integration of the melatonin-dependent photoperiodic signal onto the reproductive axis. However, it has not been possible to determine whether and how melatonin would act directly on these hypothalamic sites. Although a direct hypothalamic effect of melatonin cannot be excluded, accumulating evidence now points toward the PT as the major site for the hypothalamic integration of the melatonin signal in seasonal breeders (8, 28–30).

TSH, THYROID HORMONES, AND THE MELATONIN-DRIVEN REPRODUCTIVE ACTIVITY

In 2003, Yoshimura and colleagues made a remarkable finding that unveiled a link between the thyroid-stimulating hormone (TSH) pathway and seasonal reproduction. They showed that light-induced type 2 thyroid hormone deiodinase (Dio2) expression in the MBH and subsequent hormone conversion of thyroxine (T4) into the bioactive triiodothyronine (T3) regulate the photoperiodic response of gonads in birds (31). Since this discovery, it has been shown that TSH expression in the PT is regulated by photoperiod in a melatonin dependent manner and that TSH stimulates Dio2 expression in seasonal mammals (29, 32, 33). MT1-expressing cells in the PT synthesize TSH, and its production in the PT is strongly inhibited by the SP pattern of melatonin (34–36). Recent work moreover disclosed that melatonin regulates the photoperiodic changes in TSH expression in the PT *via* differential effects on clock gene expression and on the transcription of the co-activator EYA3 (37–39). Another primary response to photoperiodic changes in melatonin is the opposite regulation of Dio2 and type 3 thyroid hormone deiodinase (Dio3) expression in the MBH (29, 31, 33, 40–43). While Dio2 catalyzes the conversion of T4 into T3, Dio3 catalyzes the conversion of T3 to the biological inactive T2. Thus, in concert, Dio2 and Dio3 regulate the hypothalamic T4/T3 balance according to photoperiod, with a higher production of T3 in LP as compared to SP (5, 43–45). Thyroid hormones have long been known to be important for the transitions between the breeding and non-breeding states, i.e., in 1940s, thyroidectomy in starlings was reported to result in persistent breeding (46). Similarly, thyroidectomized sheep remain in the breeding state when changing from spring to anestrus and T4 replacement can reverse these effects, but interestingly, thyroidectomy displays no effect in the transition from the anestrus state to the breeding state (47–50). Moreover, T3 administration in the LP-breeding Siberian hamster blocks the SP-induced gonadal regression (44, 51, 52).

Both deiodinases are highly expressed in a population of specialized glial cells in the ependymal cell layer lining the third ventricle named tanycytes (53). Interestingly, tanycytes co-express the TSH receptor (TSHR), and recent data clearly show that activation of these receptors increases *Dio2* expression, thereby increasing levels of T3 in the MBH, in a number of seasonal mammals (5, 29, 33, 43, 54–57). In line with these observations,

a recent study shows that PT-derived TSH, in contrast to *pars distalis*-derived TSH, does not stimulate the thyroid gland, but rather acts *via* TSHR on the tanycytes (58). Altogether, these studies have unveiled a conserved photoperiodic transduction pathway explaining how the melatonin signal is integrated in the PT and transduced into a local thyroid message in the MBH (8) (Figure 1).

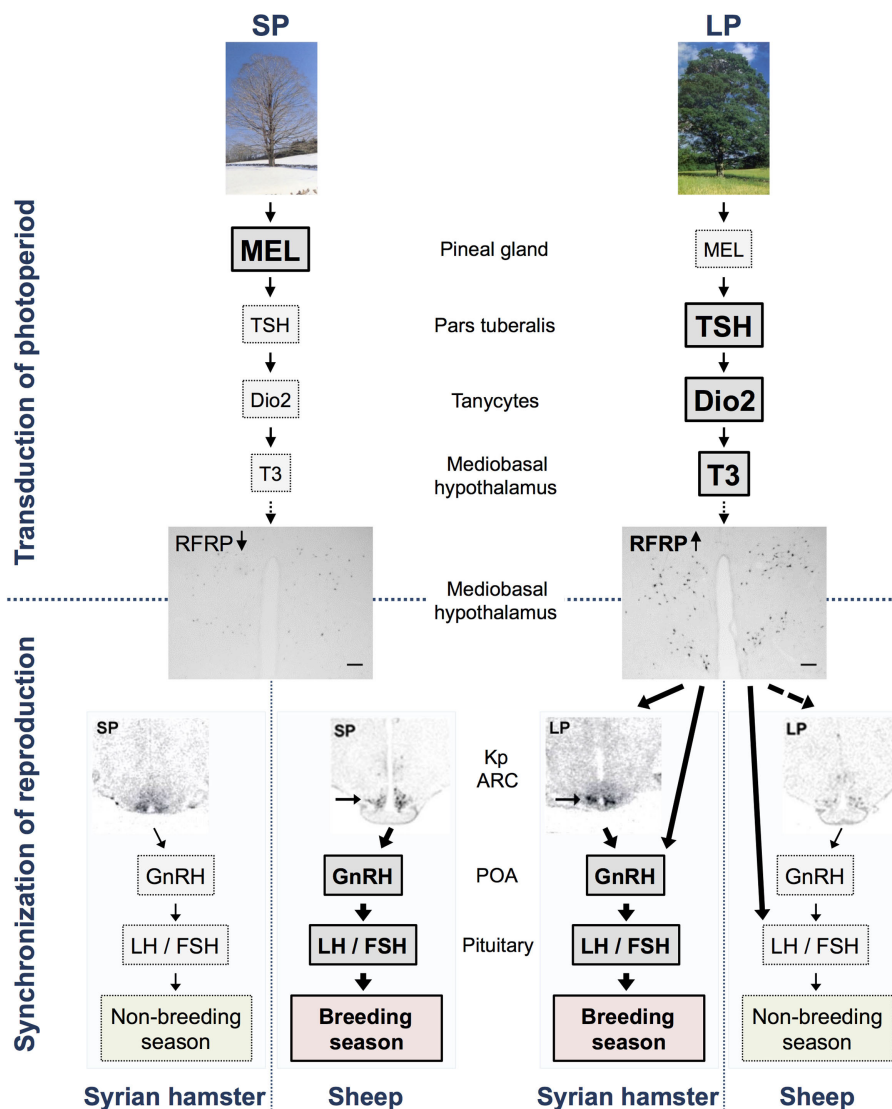


FIGURE 1 | Model of the transduction of photoperiod and seasonal regulation of the reproductive axis in long (Syrian hamsters) and short day (sheep) breeders. In short photoperiod (SP), the large production of melatonin from the pineal gland inhibits TSH synthesis in the *pars tuberalis*, whereas the lower production of melatonin in long photoperiod (LP) allows the synthesis and release of TSH. TSH is transmitted *via* TSH receptors expressed in tanycytes surrounding the third ventricle and activates the enzyme deiodinase 2 (*Dio2*). *Dio2* ultimately controls and increases the local availability of the active form of the thyroid hormone, T3, in the mediobasal hypothalamus. Subsequently, T3 regulates the expression of RFRP also in the mediobasal hypothalamus so that there is a high expression in LP and a low expression in SP in both LP and SP breeders, as demonstrated with pictures of RFRP-ir neurons in brains from Syrian hamster kept in LP and SP [scale bar 100 μ m, taken from Ref. (59)]. In Syrian hamsters, RFRP subsequently acts either directly on GnRH neurons or indirectly *via* kisspeptin (kp) neurons (indicated by arrows) to synchronize reproduction with season. In sheep, RFRP regulates the reproductive axis directly at the level of the pituitary (indicated by arrow) and possibly also directly or indirectly *via* kp neurons (indicated by dotted arrow) and/or GnRH neurons. Expression of the gene encoding kp in the ARC displays an opposite photoperiodic regulation in the two species being elevated in both LP-adapted sexually active Syrian hamsters [see arrow in picture taken from Ref. (33, 60)] and SP-adapted sexually active sheep [see arrow in picture taken from Ref. (61)]. This model does not describe specific effects of RFRP-3 reported in each species, sex, and photoperiod, and readers are referred to **Table 1** for a detailed summary.

Thyroid hormones mediate their neuroendocrine effects through still undefined hypothalamic sites since no cellular phenotyping of their receptors have been reported in the hypothalamus. Interestingly, however, a recent study found that chronic TSH administration in SP-adapted Siberian and Syrian male hamsters reactivates the reproductive axis, while at the same time increasing the expression of two known hypothalamic regulators of reproductive output, RFRP and Kp, suggesting that the melatonin signal reaches the reproductive axis *via* the TSH/thyroid hormone pathway acting on these neurons (5) rather than *via* direct hypothalamic effect as previously suggested (20).

HYPOTHALAMIC REGULATION OF SEASONAL REPRODUCTION

Hypothalamic control of the reproductive axis is commonly regulated among species through the release of GnRH from GnRH fiber terminals projecting to the median eminence. GnRH is released into the portal blood system from which it regulates the synthesis and release of pituitary gonadotropins. Despite the marked decrease of GnRH release during sexual quiescence, most seasonal species display an unchanged number and level of GnRH neurons and GnRH-immunoreactivity (ir) in the different photoperiods (62, 63). GnRH synthesis and release is regulated upstream by various signals, especially two RF-amide peptides released from, respectively, RFRP neurons in and around the dorso/ventromedial hypothalamus (DMH/VMH) and kp neurons in the anteroventral periventricular nucleus (AVPV) and medial preoptic nucleus (MPN), and in the arcuate nucleus (ARC). Kp peptides are potent stimulators of the reproductive axis and acts directly on GnRH neurons through their cognate receptor GPR54 (64, 65). Interestingly, kp expression both in the MPN/AVPV and ARC was found to be significantly downregulated by melatonin in SP-adapted sexually inactive Syrian hamsters (60, 66). In the SP-breeding sheep, kp expression is oppositely upregulated in SP (61, 67, 68), suggesting that ARC kp expression reflects the breeding state rather than the seasonal state of the animal (**Figure 1**). In both hamsters and sheep, continuous infusion of kp during sexual quiescence fully restores reproductive activity (60, 69–71), and kp neurons are thus a pivotal component between the photoperiodic signal and seasonal activation of GnRH neurons. This statement is, however, complicated by results showing a lowered ARC kp expression in LP-breeding Siberian (72, 73) and European (74) hamsters as compared to SP. While these differences in the photoperiodic regulation of ARC kp might be explained by different feedback mechanisms of sex steroids, it also suggests that these neurons are differently implicated in the photoperiodic control of reproduction from one species to another. Therefore, it seems unlikely that kp neurons are solely responsible for mediating the melatonin-dependent seasonal signal onto the reproductive axis. By contrast, increasing results demonstrate that the photoperiodic regulation of RFRP expression within the DMH/VMH is conserved among seasonal species (75), suggesting that RFRP neurons may be potential candidates for integration of the photoperiodic signal.

THE RFRP SYSTEM

RF (Arg–Phe) amide-related peptides were discovered in birds and mammals in 2000 and found to be primarily expressed in neurons located in the paraventricular nucleus (PVN) and in between the DMH and VMH in birds and rats, respectively (76, 77). In birds, the peptide was shown to inhibit gonadotropin secretion from cultured quail pituitaries and thus termed gonadotropin-inhibitory hormone (GnIH) (76). The avian GnIH precursor encodes one GnIH and two GnIH-related peptides (GnIH-RP-1 and GnIH-RP-2). GnIH and GnIH-RP-1 contain an LPLRFa motif in the C-terminal, whereas GnIH-RP-2 contains an LPQRFa motif. The mammalian gene named *RFamide-related peptide (Rfrp)* encodes RFRP-1, -2, and -3 peptides, of which RFRP-1 (containing an LPLRFa motif) and RFRP-3 (containing a C-terminal LPQRFa motif) are functional peptides (77–79). RFRP-3 and GnIH have been shown to inhibit GnRH neuron activity and gonadotropin release in several seasonal (sheep, hamster, and quail) and non-seasonal (rat and mouse) species (76, 80–87). Moreover, there is evidence of a hypophysiotropic effect of GnIH and RFRP-3 in birds and ewes, respectively, although the effect in ewe is still of controversy since in one study intravenous (iv) infusion of RFRP-3 inhibits pulsatile LH secretion in ovariectomized ewes (81), whereas two other studies find no variation in LH plasma concentrations in neither ovariectomized nor intact ewes injected either intracerebroventricular (icv) or iv with RFRP-3 (88, 89). Two recent studies reported a stimulatory effect of central administration of RFRP-3 in male Syrian and Siberian hamster, indicating that the effects of the peptide are species dependent (6, 7). Strikingly, while RFRP-3 activates the reproductive axis in male Syrian hamsters (6), the avian GnIH inhibits LH secretion in ovariectomized females (84), adding a supplementary sex difference in the effects of the peptides.

Recently, a study of Tena-Sempere and colleagues (86) reported that mouse KO for GPR147, the cognate receptor for RFRPs, does not display strong reproductive phenotypic alterations as compared to wild-type mice. Moderate changes are, however, observed in GPR147-deficient mice, as during pubertal transition, male KO mice exhibit increased LH levels, and in adulthood, FSH levels are higher in both female and male KO mice as compared to wild-type mice. Moreover, litter sizes from KO mice are slightly increased as compared to wild-type litter sizes. Interestingly, the male KO mice moreover show an increased level of kp expression in the ARC, but not in the MPN/AVPV, suggesting that in male mice, RFRP neurons provide a tonic inhibition on ARC kp neurons.

RFRP Modes and Sites of Action

RFRP neurons are mainly found in and around the DMH/VMH from where they project to multiple regions of the rodent brain. RFRP-ir fibers are found especially in the preoptic area/organum vasculosum of the lamina terminalis (POA/OVLT), MPN/AVPV, the anterior part of the SCN, PVN, anterior hypothalamus, VMH, and ARC as well as in the bed nucleus of the stria terminalis, habenular nuclei, and PVN of the thalamus (7, 59, 84). RFRP

terminals make apparent contact to 20–40% of GnRH somas in rodents and sheep (7, 67, 84, 90), and in female mice, around 20% of MPN/AVPV kp neurons and 35% of ARC kp neurons receive RFRP fiber contacts (90, 91). In mice, RFRP-3 application to brain slices inhibits the firing rate of 41% GnRH neurons and stimulates the firing rate of 12% of the GnRH neurons (82), whereas in male Syrian hamster, icv infusion of RFRP-3 induces c-FOS expression in 20–30% GnRH neurons but also in non-kisspeptinergic neurons of the ARC (6).

RFRPs bind preferentially to the G-protein-coupled receptor, GPR147 (also known as NPFF1). GPR147 has been found to couple to both stimulatory and inhibitory G protein subunits *in vitro* (92, 93), and in GPR147-transfected CHO cells, hRFRP-1 induces a maximal inhibition of a forskolin-induced cAMP accumulation, indicating that RFRP-1 might inhibit adenylate cyclase through a G α i-bound receptor complex (94). However, the exact signaling events occurring downstream of GPR147 in its natural cellular environment still remains unknown. GPR147-encoding mRNA is widely distributed in the brain; however, particular strong expression is observed in hypothalamic regions as the POA/OVLT, MPN/AVPV, SCN, PVN, anterior hypothalamus, VMH, and ARC, and outside the hypothalamus in the posterior part of the bed nucleus of the stria terminalis, habenular nuclei, and the pyramidal cell layer of the hippocampus (59, 95, 96). Interestingly, GPR147 has been shown to be expressed in 15–33% of mice GnRH neurons and a subpopulation of kp neurons in the AVPV (5–16%) and the ARC (25%) (90, 91, 97). Altogether, these studies suggest that RFRPs can act directly on these central neuroendocrine regulators of reproduction.

RFRP: A CRITICAL SWITCH BETWEEN MELATONIN AND THE REPRODUCTIVE AXIS

GnIH and Avian Seasonal Reproduction

Gonadotropin-inhibitory hormone inhibits LH release from cultured quail pituitaries (76), and to further support a direct pituitary effect of GnIH in quail, GnIH-ir fibers have been found to project to the median eminence in this species (98). Also in quail, studies have revealed that GnIH expression and release is directly regulated by melatonin acting on Mel1c receptors specifically expressed in GnIH neurons (99). In contrast to mammalian seasonal species, GnIH expression is increased by melatonin and consequently GnIH-ir expression is increased in SP as compared to LP (99). *In vitro* studies furthermore show that GnIH release has a diurnal rhythm and is increased during nighttime in quail hypothalamic explants (100). In house and song sparrows, GnIH-ir neurons are reported bigger toward the end of the breeding season (101). By contrast in wild Australia zebra finches, there is no variation in neither GnIH-ir nor *GnIH* expression between the breeding and non-breeding states (102).

RFRP in Seasonal Mammalian Species Photoperiodic Variations in the RFRP System

There are no circadian or day-to-night variations in RFRP mRNA expression in male Syrian (103) and European (74) hamsters,

respectively. RFRP expression is however strongly regulated by photoperiod and is downregulated in SP in several seasonal breeders (6, 67, 74, 75, 103, 104). Studies in male Syrian and Siberian hamsters show the SP downregulation to be melatonin dependent (7, 103). Recent findings have revealed that in female Syrian hamsters as well, RFRP expression is downregulated in SP, probably driven by the same mechanisms (59). Interestingly, RFRP expression is similarly downregulated in SP in short-day breeders such as the sheep (67, 96). This suggests that the SP pattern of circulating melatonin displays a conserved inhibition on RFRP expression independently of whether mammals are long- or short-day breeders (**Figure 1**). Importantly, the photoperiodic/melatonin regulation of RFRP expression, in contrast to kp, may not be modulated by the gonadal hormone feed back because, although RFRP neurons express sex steroid receptors (84), neither gonadectomy nor sex-hormone implants alter RFRP expression in male (103) and female (Henningsen et al., unpublished) Syrian hamsters.

We have recently shown that GPR147 mRNA levels in the Syrian hamster's brain also depends on photoperiod being downregulated in SP condition, and interestingly, this downregulation is much stronger and consistent in females as compared to males (59). In Siberian hamsters and sheep, the amount of GnRH neurons receiving RFRP fiber contacts is decreased in SP conditions (7, 81); however, in the Syrian hamster, we did not find any photoperiodic variations in numbers of RFRP-ir fibers projecting specifically to the OVLT or the ARC (59).

Species-Specific Differences in the Effects of RFRP on Seasonal Reproduction

We were the first to show that in male Syrian hamster, RFRP-3 is capable of stimulating the reproductive axis (6). In more details, RFRP-3 was found to stimulate GnRH neuronal activity, LH and FSH release, and testosterone production independently of the photoperiodic condition, although to a lesser extent in SP animals. Moreover, chronic central administration of RFRP-3 in SP-adapted male Syrian hamsters reactivated the reproductive axis *via* an increase in ARC kp expression, despite the animals being kept in SP-inhibitory conditions. The stimulatory effect of RFRP-3 observed in male Syrian hamsters fits well with the high RFRP expression in sexually active LP animals, and our data furthermore indicate that the stimulation of reproductive activity could be mediated *via* the ARC kp neurons. Thus, in the male Syrian hamster, RFRP neurons appear to integrate and transfer the seasonal input toward kp neurons. In another hamster species, the male Siberian hamster, RFRP-3 displays reverse effects depending on the photoperiodic condition, stimulating LH release in SP but decreasing LH levels in LP conditions (7). Although the mechanism underlying such photoperiod-dependent effect of RFRP-3 in this species is unknown, it might help to explain the upregulation of ARC kp in the sexually inactive SP-adapted Siberian hamsters (72, 105, 106). These observations indicate that in the two hamster species, RFRP-3 either has opposite sites of action in LP-adapted animals or is integrated differently along the hypothalamo–pituitary–gonadal axis.

Despite the controversies of RFRP's effect in sheep, one study reported that iv administration of ovine RFRP-3 peptide (also

referred to as GnIH3) inhibits LH release in ovariectomized ewe (107), which indicates that RFRPs might have a hypophysiotropic effect in ovine species, similarly to what is observed in avian species. Indeed, RFRP fibers have been shown to project to the median eminence, and RFRPs are detected in the portal blood of sheep (108, 109). As previous studies have reported, there is no evidence of peripheral effects of RFRPs on hamster's reproduction (6), thus describing another fundamental difference in how the RFRP signal is integrated in the hypothalamo–pituitary axis among mammalian seasonal species. Interestingly, the opposite effects and photoperiodic regulation of RFRP between sheep and hamsters support our hypothesis that a similar neuroendocrine pathway is conserved between LP and SP breeders with the RFRPs playing a pivotal role in adapting reproductive activity to the environment (**Figure 1**). Further analyzes are required to test this hypothesis, in particular whether the inhibitory effect of RFRP-3 account for the lower expression of kp in LP-adapted sexually inactive sheep. The reported effects of RFRP-3 in seasonal mammals are summarized in **Table 1**.

A fascinating issue is to disclose how RFRPs can have opposite seasonal and species-dependent effects. As previously mentioned, GPR147 has been found to couple to both stimulatory and inhibitory G proteins *in vitro* (92, 93), but it remains to be established if there are fundamental species-specific differences in the downstream signaling cascades after activation of the GPR147. Alternatively, it is likely that the cellular response to RFRP is conserved among species, but mediated *via* different targets. In male Syrian hamsters, acute injections of RFRP-3 induce c-Fos expression in a subset of GnRH neurons but also in an unidentified population of neurons in the ARC (6), and one can speculate whether the observed stimulatory effect of RFRP-3 in the male Syrian, but also Siberian hamsters, arises through inhibition of inhibitory interneurons, thus resulting in the stimulatory outcome. Future studies should aim at phenotyping downstream targets of RFRP in order to understand better its various effects on the reproductive axis of seasonal breeders.

Sex Differences in Seasonal Rodents

In contrast to the stimulatory effect of RFRP-3, we have observed in the male Syrian hamsters (6), central injections of the avian RFRP, GnIH, inhibits LH release in ovariectomized females (84). It should be stressed that GnIH contains a -LPLRFa motif similar to that of the mammalian RFRP-1 and not RFRP-3. However, we recently found that central RFRP-3 administration in the intact female Syrian hamster inhibits LH release similarly to the effects observed with the GnIH ortholog (Henningsson et al., unpublished). These observations add supplementary sex-specific differences in the acute effects of RFRPs, at least in Syrian hamsters. In order to understand such opposite effect of RFRP-3, we explored other potential sex differences in the Syrian hamster RFRP system. We found that the number of RFRP neurons and the intensity of the immunoreactive labeling were markedly higher in females than in males adapted to LP conditions. In SP conditions however, RFRP expression is downregulated to a similar low level in both sexes (59). The number of RFRP-ir fibers projecting specifically to the MPN/AVPV is increased in SP as compared to LP in females, but not in males. Moreover,

TABLE 1 | Overview of the *in vivo* effects of RFRP-3 on reproduction in seasonal mammals.

Species	Sex and status	Effects of RFRP-3	Reference
Siberian hamster	Male	Central administration inhibits LH release in LP Central administration stimulates LH release in SP	(7)
Syrian hamster	Male	Central acute and continuous administration stimulates LH release in LP and SP. No peripheral effect	(6)
	Female OVX	Central and peripheral administration (GnIH) inhibits LH release in LP	(84)
Sheep	Female	Peripheral injection or a 4-h perfusion has no effect on kisspeptin-mediated increase in LH in LP	(89)
		Peripheral administration inhibits LH release in SP	(107)
		Repeated peripheral injection has no effect on pulsatile LH release in LP. 24-h perfusion has no effect on E2-induced LH surge in SP	(89)
		Central and peripheral administration has no effect on LH release in SP and LP	(88)
	Female OVX	Peripheral administration inhibits E2-induced LH surge in SP	(81)
		Peripheral administration inhibits pulsatile LH release in SP	(107)
		Peripheral administration inhibits GnRH-induced LH release. RFRP-3 is detected in the portal blood in SP and LP, with higher conc. detected in LP	(109)

we found that the overall levels of GPR147 mRNA were higher in females than in males, regardless of photoperiod, and that the SP-induced downregulation of GPR147 mRNA levels were stronger in females than in males (59). A similar sex-specific difference in RFRP expression is also reported in the non-seasonal rat, where RFRP-1 expression is found to be higher in females as compared to males (110). Altogether, these findings point toward a particular importance of the RFRP system in seasonal as well as non-seasonal females.

RFRP and Circadian Changes in Female Reproductive Activity

RFRP neurons project to the MPN/AVPV (59, 84, 90) where kp neurons provide the stimulatory signal onto the GnRH neurons causing the surge of LH and thereby ovulation (111). The surge of LH requests high circulating E2 levels, as an indicator of ovarian maturation, but its timing is also gated by a circadian signal to occur at the end of the resting period (112). It has been suggested that in female mammals, RFRP neurons mediate a SCN-generated circadian output onto the MPN/AVPV kp neurons, thereby modulating the timing and generation of the LH surge (113). Indeed, in the female Syrian hamster, a decrease in RFRP expression occurs around the time of the LH surge (113), and in rat and ewes, RFRP expression is similarly reduced during the preovulatory period

(107, 110). Interestingly, a recent study showed that SCN-derived vasoactive intestinal peptide (VIP)-ergic terminal fibers projections are found in the area of where RFRP neurons are expressed in the female Syrian hamster and more importantly that central VIP administration markedly suppress RFRP cellular activity in the evening, but not in the morning (114). Altogether, these data point toward a specific circadian rhythm in RFRP expression and release in females, adding a supplementary role of RFRP in regulating reproductive activity in seasonal female species.

CONCLUSION

Over the past nearly two decades, RFRPs have been extensively studied for their putative involvement in the regulation of the reproductive axis (87). Initially, RFRPs were thought to act with a similar inhibitory effect as the avian ortholog GnIH in all species and is still widely referred to as GnIH, despite its well-documented stimulatory effect in Siberian and Syrian hamster species (6, 7). These recent findings have challenged the conception of a conserved role of RFRPs throughout species and underline the necessity to delineate its effects in one species, sex, and physiological condition, such as season.

The marked downregulation of RFRP expression observed in seasonal mammals, independently of sex, species, and breeding

behavior, provides evidence for a distinct and conserved role of this peptide in the integration of the photoperiodic melatonin signal *via* a TSH-dependent regulation of thyroid hormone locally in the MBH (5, 8, 75) (**Figure 1**).

In the Syrian hamster, RFRP has opposite effects in females and males, and there are strong sex-specific differences in the RFRP system manifested by a higher expression and stronger photoperiodic variations in the RFRP system in females as compared to males. Few studies have investigated the regulation and role of the RFRP system in female reproduction; however, findings so far strongly suggest that RFRP neurons play a critical role in female reproduction in regards to the timing of both daily and seasonal synchronization of their reproductive activity.

AUTHOR CONTRIBUTIONS

JH has written a large part of the review; FG and VS have supervised the writing, corrected the text, and helped in the design of the figure.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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